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| <p>(54) Title: PRESERVATIVE SYSTEMS WITH ENHANCED ANTIMICROBIAL ACTIVITY<br/>(57) Abstract<br/><br/>A preservative system includes a non-irritating level of a quaternary ammonium compound such as benzalkonium chloride or cetylpyridinium chloride and either a paraben, hydrophobic chelator, or alcohol compound. The combination of the two compounds provides for synergistic killing activity in terms of efficacy against test bacteria, hard to kill bacteria, and adapted bacteria, as well as yeasts and molds, and in terms of speed of kill.</p>    |           |   |

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## PRESERVATIVE SYSTEMS WITH ENHANCED ANTIMICROBIAL ACTIVITY

### DESCRIPTION

5

#### BACKGROUND OF THE INVENTION

##### *Field of the Invention*

10           The invention is generally related to  
preservatives used in solutions such as nasal  
sprays, eye and contact lens solutions, shampoos,  
liquid cold formulations, and other health care  
products. More particularly, the invention is  
15           directed to particular combinations of compounds  
which have synergistic antimicrobial activity and  
enhanced speed of kill.

##### *Description of the Prior Art*

20

          Quaternary ammonium compounds such as  
benzalkonium chloride (BAK) and cetyl pyridinium  
chloride (CPC) have been used for many years as  
preservatives. For example, U.S. Patents 2,694,663  
25           and 2,666,010 to Stayner describe the use of  
quaternary ammonium compounds as germicidal and  
microbicidal agents, and U.S. Patent 2,295,505 to  
Shelton discloses the use of cetyl quaternary  
ammonium compounds for controlling microorganisms.  
30           Even today, BAK and CPC continue to be the  
antimicrobial agents of choice in a wide variety of  
solutions. For example, U.S. Patent 5,017,617 to  
Kihara et al. and U.S. Patent 4,474,748 describe a

disinfectant solution and a medication, respectively, and both patents note that BAK and CPC can be employed in solution as antimicrobial agents.

5           A significant problem with BAK and CPC is that they can be very irritating to eyes or other tissues when used in a solution at concentrations greater than 0.01% by weight of the solution. Yet, BAK and CPC are ineffective against some  
10 microorganisms when employed at lower concentrations (e.g., less than 50 ppm), often leading to product contamination. Hence, prior art formulations which employ BAK and CPC have an inherent trade-off between irritability and  
15 effectiveness. Other compounds which have commonly been employed as preservatives such as ethylenediaminetetraacetic acid (EDTA), benzyl alcohol (BA), thimerosal, chlorhexidine gluconate (CHG), and polyaminopropyl biguanide (PAPB), also  
20 cause irritation problems. The desire to reduce concentrations of preservatives to less irritating levels is counterbalanced not only by an increased risk of contamination, but by increasing regulatory pressure to have preservative systems that kill  
25 microorganisms faster. Until this invention, it has proven difficult to lower preservative levels while maintaining or increasing the killing effectiveness of the preservative including the rate of kill.

30           Not only do compositions with highly irritable preservatives have difficulty in gaining market acceptance, they also pose significant health problems. Recently, many investigators in the

contact lens field have suggested using alternatives to BAK because of irritation to the patient's eye. Nasal sprays, liquid cold formulations, shampoos, and other health care products would also suffer from irritability problems.

The performance of a preservative system is difficult to predict and must generally be proven empirically. A preservative system's ability to pass the U.S. Pharmacopeia (USP) Preservative Effectiveness Test does not guarantee that the product will remain free of contamination, and such contamination is common with some products that have passed the USP test. In-use testing and the use of "tough" product isolates should be employed to help ensure that a preservative system will be effective under real world conditions.

#### SUMMARY OF THE INVENTION

It is an object of this invention to provide preservative systems with enhanced microbial killing activity, including a more rapid rate of kill.

According to the invention, it has been determined that using certain compounds in conjunction with quaternary ammonium compounds yields a preservative combination with synergistic microorganism killing activity. The quaternary ammonium compounds contemplated by this invention include BAK and CPC as well as other related quaternary ammonium compounds including substituted benzalkonium compounds such as

alkyldimethylethylbenzylammonium chloride or heteroaromatic ammonium salts, twin chain quats like dioctyl dimethyl ammonium bromide, bisquaternary ammonium salts such as triclobisonium chloride, polymeric quaternary ammonium compounds such as polyquaternium, and tri-quaternary phosphate esters such as monoquat PTC, etc. Combinations of two or more of the quaternary ammonium compounds could also be employed in the preservative systems of this invention. The compounds used in combination with quaternary ammonium compounds include methyl paraben and other parabens such as ethyl, propyl and butyl paraben, alcohols such as benzyl alcohol and phenylethyl alcohol, and hydrophobic chelators such as phenanthroline and its derivatives such as methyl, nitro, and chlorophenanthroline, thenoyltrifluoroacetone, hydroxyquinoline and derivatives, bipyridine, picolinic acid, and dipicolinic acid.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

Experiments have been conducted which demonstrate synergistic antimicrobial activity and enhanced speed of kill for solutions which include a preservative system having a quaternary ammonium compound and a paraben, alcohol, or hydrophobic chelator compound.

The preservative systems were tested against *Staphylococcus aureus* 6538, which is a standard tester strain approved for preservative efficacy

testing by the USP and British Pharmacopeia (BP). The preservative systems were also tested against *Pseudomonas cepacia* 25416 and *Serratia marcescens* 48, which are rigorous or "tough" environmental organisms. *P. cepacia* and *S. marcescens* represent the most potent challenge to preserved formulations and, in recent years, have been common causes of product contamination. Hence, effective preservatives will need to kill these organisms even though they are not currently required for standardized testing. Some testing of the preservative systems against "adapted" *S. marcescens* has been performed. An "adapted" bacterium is one that has been grown in the presence of low levels of an antimicrobial agent and can survive in solutions containing the antimicrobial agent at levels that would kill unadapted bacteria of the same strain. The ability to kill "adapted" *S. marcescens* demonstrates the superior efficacy of the preservative systems contemplated by this invention. In addition, some testing of the preservative systems against *Candida albicans*, *Pseudomonas aeruginosa*, and *Aspergillus niger*, all of which are ubiquitous environmental organisms and are used as standard USP and BP test strains, has been performed.

Testing was performed according to the USP XXII and BP standard methods for determining preservative efficacy. In summary, solutions containing the preservative were inoculated with  $10^6$  test bacteria per milliliter ( $10^6$ /ml), and at the time intervals of six hours, one day, three days, and seven days after inoculation, 0.1 ml of

solution was spread plated onto a neutralizing agar (one that neutralizes the effects of any carryover preservative) and the viable microorganisms were counted. The test procedure demonstrates both the effectiveness of the preservative system and the speed of kill. In Great Britain, there is a requirement that the preservative system for contact lenses have a three log kill of the test microorganism within six hours and that no viable microorganisms be present after twenty four hours contact with the preservative. The U.S. also has a regulatory trend towards more rapid killing.

The lowest BAK concentration generally used in current contact lens solutions is 40 ppm. Products containing this level of BAK can be contaminated by tough *Pseudomonas* species and *S. marcescens*. Many eye care solutions use as much as 100 ppm BAK to increase antimicrobial activity; however, this level of BAK causes eye irritation.

Methyl paraben (MP) is a preservative that is commonly used in cosmetics and food products at concentrations ranging from 0.1 to 1.0%. Methyl paraben is not currently used in contact lens solutions.

Tables 1 through 5 provide test data which demonstrate the antimicrobial effectiveness of a preservative system which includes a combination of MP and BAK in aqueous solutions of phosphate buffered saline (PBS, 0.025M potassium phosphate, 0.7% wt/wt NaCl) at near neutral pH conditions ranging from pH 6 to pH 8.



TABLE 1  
Antimicrobial Effectiveness of MP + BAK  
combinations against *P. cepacia* 25416

| pH 7.0 PBS |                |       |                             |                     |                     |                     |
|------------|----------------|-------|-----------------------------|---------------------|---------------------|---------------------|
| 5          | methyl paraben |       | Benzalkonium chloride (BAK) |                     |                     |                     |
|            | in ppm         |       |                             |                     |                     |                     |
|            | % wt/wt        | Time  | 0                           | 5                   | 10                  | 20                  |
| 10         | 0              | 6hr   | 5.3x10 <sup>5</sup>         | 7.7x10 <sup>5</sup> | 6.8x10 <sup>5</sup> | 3.3x10 <sup>5</sup> |
|            |                | 1day  | 1.7x10 <sup>6</sup>         | 7.8x10 <sup>5</sup> | 7.6x10 <sup>5</sup> | 7.4x10 <sup>5</sup> |
|            |                | 3days | 2.0x10 <sup>6</sup>         | 1.3x10 <sup>6</sup> | 1.8x10 <sup>6</sup> | 6.4x10 <sup>5</sup> |
|            |                | 7days | 6.2x10 <sup>5</sup>         | 1.5x10 <sup>6</sup> | 3.3x10 <sup>6</sup> | 5.8x10 <sup>5</sup> |
| 15         | 0.05           | 6hr   | 3.8x10 <sup>5</sup>         | 4.4x10 <sup>4</sup> | 3.4x10 <sup>2</sup> | 1.0x10 <sup>1</sup> |
|            |                | 1day  | 5.9x10 <sup>5</sup>         | 2.3x10 <sup>4</sup> | 2.0x10 <sup>1</sup> | <10                 |
|            |                | 3days | 1.0x10 <sup>4</sup>         | 1.4x10 <sup>3</sup> | <10                 | <10                 |
|            |                | 7days | 1.7x10 <sup>5</sup>         | 1.6x10 <sup>6</sup> | <10                 | <10                 |
| 20         | 0.1            | 6hr   | 5.0x10 <sup>5</sup>         | <10                 | <10                 | <10                 |
|            |                | 1day  | 1.7x10 <sup>3</sup>         | <10                 | <10                 | <10                 |
|            |                | 3days | <10                         | <10                 | <10                 | <10                 |
|            |                | 7days | <10                         | <10                 | <10                 | <10                 |
| pH 6.0 PBS |                |       |                             |                     |                     |                     |
| 25         | 0.1            | 6hr   | 5.4x10 <sup>5</sup>         | 3.0x10 <sup>1</sup> | <10                 | <10                 |
|            |                | 1day  | 6.9x10 <sup>4</sup>         | <10                 | <10                 | <10                 |
|            |                | 3days | 8.0x10 <sup>1</sup>         | <10                 | <10                 | <10                 |
|            |                | 7days | <10                         | <10                 | <10                 | <10                 |
| pH 8.0 PBS |                |       |                             |                     |                     |                     |
| 30         | 0.1            | 6hr   | 4.4x10 <sup>5</sup>         | <10                 | <10                 | <10                 |
|            |                | 1day  | 1.9x10 <sup>4</sup>         | <10                 | <10                 | <10                 |
|            |                | 3days | <10                         | <10                 | <10                 | <10                 |
|            |                | 7days | <10                         | <10                 | <10                 | <10                 |

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TABLE 2  
Antimicrobial Effectiveness of MP + BAK  
combinations against *S. aureus* 6538

| pH 7.0 PBS |                |       |                             |                     |     |     |
|------------|----------------|-------|-----------------------------|---------------------|-----|-----|
| 5          | methyl paraben |       | Benzalkonium chloride (BAK) |                     |     |     |
|            | in ppm         |       |                             |                     |     |     |
|            | % wt/wt        | Time  | 0                           | 5                   | 10  | 20  |
|            | 0              | 6hr   | 7.6x10 <sup>5</sup>         | 5.6x10 <sup>2</sup> | <10 | <10 |
|            |                | 1day  | 6.0x10 <sup>5</sup>         | 3.0x10 <sup>1</sup> | <10 | <10 |
|            |                | 3days | 3.6x10 <sup>4</sup>         | <10                 | <10 | <10 |
|            |                | 7days | 3.0x10 <sup>4</sup>         | <10                 | <10 | <10 |
| 10         |                |       |                             |                     |     |     |
|            | 0.05           | 6hr   | 9.0x10 <sup>4</sup>         | 6.2x10 <sup>2</sup> | <10 | <10 |
|            |                | 1day  | 9.0x10 <sup>1</sup>         | <10                 | <10 | <10 |
|            |                | 3days | <10                         | <10                 | <10 | <10 |
|            |                | 7days | <10                         | <10                 | <10 | <10 |
| 15         |                |       |                             |                     |     |     |
|            | 0.1            | 6hr   | 5.3x10 <sup>5</sup>         | <10                 | <10 | <10 |
|            |                | 1day  | 1.2x10 <sup>4</sup>         | <10                 | <10 | <10 |
|            |                | 3days | <10                         | <10                 | <10 | <10 |
|            |                | 7days | <10                         | <10                 | <10 | <10 |
| 20         |                |       |                             |                     |     |     |
| pH 6.0 PBS |                |       |                             |                     |     |     |
|            | 0.1            | 6hr   | 7.1x10 <sup>4</sup>         | 2.3x10 <sup>2</sup> | <10 | <10 |
|            |                | 1day  | 2.6x10 <sup>2</sup>         | <10                 | <10 | <10 |
|            |                | 3days | <10                         | <10                 | <10 | <10 |
|            |                | 7days | <10                         | <10                 | <10 | <10 |
| 25         |                |       |                             |                     |     |     |
| pH 8.0 PBS |                |       |                             |                     |     |     |
|            | 0.1            | 6hr   | 1.2x10 <sup>4</sup>         | <10                 | <10 | <10 |
|            |                | 1day  | <10                         | <10                 | <10 | <10 |
|            |                | 3days | <10                         | <10                 | <10 | <10 |
|            |                | 7days | <10                         | <10                 | <10 | <10 |
| 30         |                |       |                             |                     |     |     |

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TABLE 3  
Antimicrobial Effectiveness of MP + BAK + 0.1%  
EDTA

| Combination against <i>P. cepacia</i> 25416 |                |       |                     |                             |                     |                     |
|---|----------------|-------|---------------------|-----------------------------|---------------------|---------------------|
| 5   | pH 7.0 PBS     |       |                     |                             |                     |                     |
|   | methyl paraben |       |                     | Benzalkonium chloride (BAK) |                     |                     |
|   | in ppm         |       |                     |                             |                     |                     |
|   | % wt/wt        | Time  | 0                   | 5                           | 10                  | 20                  |
| 10  | 0              | 6hr   | 6.9x10 <sup>5</sup> | 5.2x10 <sup>5</sup>         | 6.4x10 <sup>5</sup> | 7.5x10 <sup>5</sup> |
|   |                | 1day  | 1.3x10 <sup>6</sup> | 1.2x10 <sup>6</sup>         | 1.0x10 <sup>6</sup> | 1.2x10 <sup>6</sup> |
|   |                | 3days | 2.0x10 <sup>6</sup> | 1.4x10 <sup>6</sup>         | 1.5x10 <sup>6</sup> | 2.9x10 <sup>6</sup> |
|   |                | 7days | 9.8x10 <sup>5</sup> | 1.8x10 <sup>6</sup>         | 1.2x10 <sup>6</sup> | 1.4x10 <sup>6</sup> |
| 15  | 0.05           | 6hr   | 8.4x10 <sup>5</sup> | 5.0x10 <sup>4</sup>         | 6.0x10 <sup>3</sup> | <10                 |
|   |                | 1day  | 5.3x10 <sup>5</sup> | 1.5x10 <sup>4</sup>         | 1.1x10 <sup>3</sup> | <10                 |
|   |                | 3days | 2.0x10 <sup>4</sup> | 2.0x10 <sup>3</sup>         | 2.0x10 <sup>1</sup> | <10                 |
|   |                | 7days | 9.6x10 <sup>2</sup> | <10                         | <10                 | <10                 |
| 20  | 0.1            | 6hr   | 1.9x10 <sup>5</sup> | <10                         | <10                 | <10                 |
|   |                | 1day  | 8.6x10 <sup>4</sup> | <10                         | <10                 | <10                 |
|   |                | 3days | 3.0x10 <sup>3</sup> | <10                         | <10                 | <10                 |
|   |                | 7days | <10                 | <10                         | <10                 | <10                 |
| pH 6.0 PBS                                  |                |       |                     |                             |                     |                     |
| 25  | 0.1            | 6hr   | 1.7x10 <sup>5</sup> | 1.4x10 <sup>3</sup>         | <10                 | <10                 |
|   |                | 1day  | 3.2x10 <sup>4</sup> | <10                         | <10                 | <10                 |
|   |                | 3days | 1.6x10 <sup>3</sup> | <10                         | <10                 | <10                 |
|   |                | 7days | <10                 | <10                         | <10                 | <10                 |
| pH 8.0 PBS                                  |                |       |                     |                             |                     |                     |
| 30  | 0.1            | 6hr   | 5.8x10 <sup>5</sup> | 2.0x10 <sup>1</sup>         | <10                 | <10                 |
|   |                | 1day  | 8.0x10 <sup>3</sup> | <10                         | <10                 | <10                 |
|   |                | 3days | <10                 | <10                         | <10                 | <10                 |
|   |                | 7days | <10                 | <10                         | <10                 | <10                 |

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TABLE 4  
Antimicrobial Effectiveness of MP + BAK + 0.1%  
EDTA  
combinations against *S. aureus* 6538

|    |                |       |                             |                     |                     |                     |
|----|----------------|-------|-----------------------------|---------------------|---------------------|---------------------|
| 5  | pH 7.0 PBS     |       |                             |                     |                     |                     |
|    | methyl paraben |       | Benzalkonium chloride (BAK) |                     |                     |                     |
|    | in ppm         |       |                             |                     |                     |                     |
|    | % wt/wt        | Time  | 0                           | 5                   | 10                  | 20                  |
|    | 0              | 6hr   | 9.8x10 <sup>5</sup>         | 1.9x10 <sup>5</sup> | 3.5x10 <sup>4</sup> | 5.0x10 <sup>1</sup> |
| 10 |                | 1day  | 8.5x10 <sup>5</sup>         | 7.0x10 <sup>3</sup> | 6.0x10 <sup>1</sup> | <10                 |
|    |                | 3days | 5.2x10 <sup>5</sup>         | <10                 | <10                 | <10                 |
|    |                | 7days | 1.6x10 <sup>5</sup>         | <10                 | <10                 | <10                 |
|    | 0.05           | 6hr   | 5.6x10 <sup>5</sup>         | 1.5x10 <sup>5</sup> | <10                 | <10                 |
| 15 |                | 1day  | 6.3x10 <sup>5</sup>         | <10                 | <10                 | <10                 |
|    |                | 3days | 3.6x10 <sup>4</sup>         | <10                 | <10                 | <10                 |
|    |                | 7days | 4.6x10 <sup>2</sup>         | <10                 | <10                 | <10                 |
|    | 0.1            | 6hr   | 2.6x10 <sup>5</sup>         | 4.0x10 <sup>2</sup> | <10                 | <10                 |
| 20 |                | 1day  | 1.9x10 <sup>4</sup>         | <10                 | <10                 | <10                 |
|    |                | 3days | <10                         | <10                 | <10                 | <10                 |
|    |                | 7days | <10                         | <10                 | <10                 | <10                 |
|    | pH 6.0 PBS     |       |                             |                     |                     |                     |
|    | 0.1            | 6hr   | 3.0x10 <sup>3</sup>         | 1.0x10 <sup>4</sup> | 1.3x10 <sup>2</sup> | <10                 |
| 25 |                | 1day  | 7.0x10 <sup>1</sup>         | <10                 | <10                 | <10                 |
|    |                | 3days | <10                         | <10                 | <10                 | <10                 |
|    |                | 7days | <10                         | <10                 | <10                 | <10                 |
|    | pH 8.0 PBS     |       |                             |                     |                     |                     |
|    | 0.1            | 6hr   | 3.5x10 <sup>5</sup>         | 5.0x10 <sup>1</sup> | <10                 | <10                 |
| 30 |                | 1day  | 3.0x10 <sup>3</sup>         | <10                 | <10                 | <10                 |
|    |                | 3days | <10                         | <10                 | <10                 | <10                 |
|    |                | 7days | <10                         | <10                 | <10                 | <10                 |

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TABLE 5  
Antimicrobial Effectiveness of MP + BAK + 0.1%  
EDTA  
combinations against adapted and unadapted *S.*  
*marcescens* 48

|    |                                |       |                     |                             |                     |                     |  |
|----|--------------------------------|-------|---------------------|-----------------------------|---------------------|---------------------|--|
| 5  | marcescens 48                  |       |                     |                             |                     |                     |  |
|    | pH 7.0 PBS                     |       |                     |                             |                     |                     |  |
|    | Unadapted <i>S. marcescens</i> |       |                     |                             |                     |                     |  |
|    | 48                             |       |                     |                             |                     |                     |  |
|    | methyl paraben                 |       |                     | Benzalkonium chloride (BAK) |                     |                     |  |
| 10 | in ppm                         |       |                     |                             |                     |                     |  |
|    | % wt/wt                        | Time  | 0                   | 5                           | 10                  | 20                  |  |
|    | 0                              | 6hr   | 9.5x10 <sup>5</sup> | 7.4x10 <sup>5</sup>         | 4.2x10 <sup>5</sup> | 1.8x10 <sup>5</sup> |  |
|    |                                | 1day  | 1.3x10 <sup>6</sup> | 6.2x10 <sup>5</sup>         | 7.3x10 <sup>5</sup> | 4.4x10 <sup>5</sup> |  |
|    |                                | 3days | 1.5x10 <sup>6</sup> | 5.5x10 <sup>5</sup>         | 6.2x10 <sup>5</sup> | 4.8x10 <sup>5</sup> |  |
| 15 |                                |       |                     |                             |                     |                     |  |
|    | 0.1                            | 6hr   | 3.7x10 <sup>5</sup> | <10                         | <10                 | <10                 |  |
|    |                                | 1day  | 1.7x10 <sup>5</sup> | <10                         | <10                 | <10                 |  |
|    |                                | 3days | 6.4x10 <sup>4</sup> | <10                         | <10                 | <10                 |  |
|    | pH 7.0 PBS                     |       |                     |                             |                     |                     |  |
| 20 | Adapted <i>S. marcescens</i>   |       |                     |                             |                     |                     |  |
|    | 48                             |       |                     |                             |                     |                     |  |
|    | methyl paraben                 |       |                     | Benzalkonium chloride (BAK) |                     |                     |  |
|    | in ppm                         |       |                     |                             |                     |                     |  |
|    | % wt/wt                        | Time  | 0                   | 5                           | 10                  | 20                  |  |
| 25 | 0                              | 6hr   | 1.2x10 <sup>6</sup> | 7.6x10 <sup>5</sup>         | 7.4x10 <sup>5</sup> | 7.0x10 <sup>5</sup> |  |
|    |                                | 1day  | 1.3x10 <sup>6</sup> | 5.0x10 <sup>5</sup>         | 4.4x10 <sup>5</sup> | 5.5x10 <sup>5</sup> |  |
|    |                                | 3days | 1.2x10 <sup>6</sup> | 5.3x10 <sup>5</sup>         | 4.8x10 <sup>5</sup> | 6.1x10 <sup>5</sup> |  |
|    |                                |       |                     |                             |                     |                     |  |
|    | 0.1                            | 6hr   | 1.0x10 <sup>5</sup> | <10                         | <10                 | <10                 |  |
| 30 |                                | 1day  | 7.2x10 <sup>4</sup> | <10                         | <10                 | <10                 |  |
|    |                                | 3days | 5.0x10 <sup>4</sup> | <10                         | <10                 | <10                 |  |

The indication "<10" in Tables 1-5 indicates that no viable microorganisms were identified when a 0.1 ml aliquot was plated onto the agar.

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Table 1 shows that solutions containing BAK alone, at concentrations as high as 20 ppm, were not effective in killing the tough *P. cepacia* microorganism. Solutions having only MP at  
5 higher concentrations (0.1%) were effective against *P. cepacia*; however, the speed of kill with these solutions was unacceptable. Specifically, it took one to three days to  
10 achieve a three log reduction in viable microorganisms with MP alone, whereas it

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is preferable to have a three log reduction after only six hours contact with the preservative.

In contrast, Table 1 shows that solutions containing a combination of BAK and MP had an enhanced, synergistic killing capacity. Note particularly that in solution at pH 7, a three log reduction in viable *P. cepacia* occurred after six hours when the solution had as little as 0.05% MP and 10 ppm BAK. At both pH 6 and pH 8, a three log reduction occurred after six hours in a solution containing 0.1 % MP and as little as 5 ppm BAK.

Table 2 shows that the MP and BAK combination was highly effective at killing *S. aureus*.

Tables 3 and 4 show that adding EDTA to the preservative combination of MP and BAK has only a very limited effect on microorganism killing activity. However, EDTA is often added to preservative combinations because of its known effectiveness against *P. aeruginosa*. Comparing Tables 1 and 3, it can be seen that a three log kill of *P. cepacia* after six hours exposure was achieved with solutions containing 0.05% MP and 10 ppm BAK, with and without 0.1% EDTA. In addition, it is noted from Table 3 that a combination of 0.1% wt/wt EDTA and even 20 ppm BAK, without any MP, was ineffective against the tough *P. cepacia* bacterium. While Table 4 shows that a solution containing 0.1% EDTA and 20 ppm BAK, and no MP, was effective at killing *S. aureus* (e.g., three log reduction) after six hours, it should be understood that *S. aureus* is a far easier bacterium to kill than *P. cepacia* and that, because *P. cepacia* represents a serious contamination problem, the killing capacity of the

preservative for *P. cepacia* is an important measure of the preservative's effectiveness. Furthermore, Table 2 demonstrates that solutions with low levels of BAK, without EDTA or MP, are capable of killing *S. aureus* at an effective rate.

The "adapted" *S. marcescens* strains reported in Table 5 were produced by growth of the bacteria in a 10% dilution of 0.1%MP + 0.1% EDTA + 20 ppm BAK in 1 g/l trypticase soy broth (TSB). Table 5 demonstrates that preservative systems containing a combination of MP and BAK and EDTA are highly effective against both adapted and unadapted strains of *S. marcescens*. As little as 5 ppm BAK were required in the preservative system to achieve a three log kill of the adapted and unadapted *S. marcescens* within six hours. Neither 0.1% methyl paraben nor 20 ppm BAK showed any significant effect when used alone.

The data in Tables 1 through 5 demonstrate that the combination of MP and BAK provides for a preservative system with a synergistically enhanced killing capacity. While Tables 1 through 5 show test results for formulations having a pH between pH6 and pH 8, similar results would be expected within the pH range of pH 5 to pH 9.

Thenoyltrifluoroacetone (TTFA) is a hydrophobic chelating compound. Prior to this invention, no preservative activity was known to exist for TTFA. Table 6 shows that there is a synergistic killing activity against a variety of microorganisms when TTFA is used in combination with BAK. The bacteria and fungi tested against this combination include *A. niger* 19606, *S. aureus*



6538, *S. marcescens* 48, *P. cepacia* 25416, *P. aeruginosa* 9027, and *C. albicans* 10231. Like *S. aureus*; *A. niger*, *P. aeruginosa*, and *C. albicans* are standard USP and BP preservative efficacy test microorganisms. As discussed above, *S. marcescens* and *P. cepacia* are bacteria which are difficult to kill and are the source of contamination in many health care products.

TABLE 6

Antimicrobial Effectiveness of TTFA used in Combination with BAK and EDTA

|             |                      | Viability         |                   |                   |                   |
|-------------|----------------------|-------------------|-------------------|-------------------|-------------------|
| Formulation | Organism             | 6hr               | 1day              | 3days             | 7days             |
| PBS at      | <i>A. niger</i>      | $1.4 \times 10^5$ | $1.8 \times 10^5$ | $1.8 \times 10^5$ | $1.0 \times 10^5$ |
| pH 7.0      | <i>S. aureus</i>     | $1.5 \times 10^6$ | $1.2 \times 10^6$ | $2.8 \times 10^5$ | $3.4 \times 10^3$ |
| with        | <i>S. marcescens</i> | $5.4 \times 10^5$ | $8.8 \times 10^5$ | $1.0 \times 10^6$ | $8.5 \times 10^5$ |
| 1.0%        | <i>P. cepacia</i>    | $1.2 \times 10^6$ | $1.2 \times 10^6$ | $1.1 \times 10^6$ | $2.0 \times 10^6$ |
| EDTA        | <i>P. aeruginosa</i> | $1.5 \times 10^6$ | $1.5 \times 10^6$ | $1.4 \times 10^6$ | $9.3 \times 10^6$ |
|             | <i>C. albicans</i>   | $5.4 \times 10^5$ | $6.0 \times 10^5$ | $6.4 \times 10^5$ | $2.4 \times 10^5$ |
| PBS at      | <i>A. niger</i>      | $9.2 \times 10^2$ | <10               | <10               | <10               |
| pH 7.0      | <i>S. aureus</i>     | $6.0 \times 10^5$ | $2.0 \times 10^3$ | <10               | <10               |
| with        | <i>S. marcescens</i> | $8.6 \times 10^5$ | $5.4 \times 10^5$ | $8.4 \times 10^5$ | $6.6 \times 10^5$ |
| 10 ppm      | <i>P. cepacia</i>    | $1.2 \times 10^6$ | $2.6 \times 10^6$ | $2.6 \times 10^6$ | $2.1 \times 10^6$ |
| BAK         | <i>P. aeruginosa</i> | $7.6 \times 10^5$ | $1.2 \times 10^6$ | $1.5 \times 10^6$ | $6.1 \times 10^5$ |
|             | <i>C. albicans</i>   | $4.0 \times 10^5$ | $5.6 \times 10^2$ | $3.6 \times 10^2$ | $4.0 \times 10^1$ |
| PBS at      | <i>A. niger</i>      | $5.0 \times 10^3$ | $3.0 \times 10^1$ | $1.0 \times 10^1$ | $1.0 \times 10^1$ |
| pH 7 with   | <i>S. aureus</i>     | $7.2 \times 10^5$ | $1.2 \times 10^5$ | $3.0 \times 10^1$ | <10               |
| 0.1%        | <i>S. marcescens</i> | $9.3 \times 10^5$ | $7.0 \times 10^5$ | $1.1 \times 10^6$ | $8.4 \times 10^5$ |
| EDTA +      | <i>P. cepacia</i>    | $9.3 \times 10^5$ | $1.1 \times 10^6$ | $1.2 \times 10^6$ | $1.9 \times 10^6$ |
| 10 ppm      | <i>P. aeruginosa</i> | <10               | <10               | <10               | <10               |
| BAK         | <i>C. albicans</i>   | $4.5 \times 10^5$ | $2.1 \times 10^4$ | $4.5 \times 10^2$ | <10               |
| PBS         | <i>A. niger</i>      | $9.3 \times 10^4$ | $7.2 \times 10^4$ | $2.6 \times 10^4$ | $4.0 \times 10^3$ |
| at pH 7     | <i>S. aureus</i>     | $6.1 \times 10^4$ | $1.3 \times 10^4$ | $5.0 \times 10^1$ | <10               |

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|    |           |                      |                   |                   |                   |                   |
|----|-----------|----------------------|-------------------|-------------------|-------------------|-------------------|
|    | with      | <i>S. marcescens</i> | $2.0 \times 10^3$ | <10               | <10               | <10               |
|    | 0.1%      | <i>P. cepacia</i>    | $2.4 \times 10^5$ | $2.3 \times 10^3$ | $1.6 \times 10^3$ | $1.3 \times 10^4$ |
|    | TTFA      | <i>P. aeruginosa</i> | <10               | <10               | <10               | <10               |
|    |           | <i>C. albicans</i>   | $6.3 \times 10^2$ | $5.0 \times 10^1$ | <10               | <10               |
| 5  | PBS at    | <i>A. niger</i>      | $9.5 \times 10^4$ | $3.9 \times 10^4$ | $2.0 \times 10^3$ | $2.7 \times 10^2$ |
|    | pH 7 with | <i>S. aureus</i>     | $1.2 \times 10^6$ | $4.6 \times 10^5$ | $2.4 \times 10^4$ | <10               |
|    | 0.1%      | <i>S. marcescens</i> | $3.8 \times 10^5$ | $1.5 \times 10^5$ | $5.0 \times 10^3$ | <10               |
|    | TTFA +    | <i>P. cepacia</i>    | $1.6 \times 10^5$ | $7.0 \times 10^3$ | <10               | <10               |
| 10 | 0.1%      | <i>P. aeruginosa</i> | $1.0 \times 10^2$ | <10               | <10               | <10               |
|    | EDTA      | <i>C. albicans</i>   | $3.0 \times 10^5$ | $2.6 \times 10^5$ | $2.9 \times 10^4$ | <10               |
|    | PBS       | <i>A. niger</i>      | $2.2 \times 10^4$ | <10               | <10               | <10               |
|    | at pH 7   | <i>S. aureus</i>     | $1.2 \times 10^3$ | <10               | <10               | <10               |
| 15 | with 0.1% | <i>S. marcescens</i> | $7.0 \times 10^1$ | <10               | <10               | <10               |
|    | TTFA +    | <i>P. cepacia</i>    | <10               | <10               | <10               | <10               |
|    | 10 ppm    | <i>P. aeruginosa</i> | <10               | <10               | <10               | <10               |
|    |           | <i>C. albicans</i>   | <10               | <10               | <10               | <10               |
| 20 | PBS at    | <i>A. niger</i>      | $3.1 \times 10^2$ | <10               | <10               | <10               |
|    | pH 7 with | <i>S. aureus</i>     | <10               | <10               | <10               | <10               |
|    | 0.1%      | <i>S. marcescens</i> | <10               | <10               | <10               | <10               |
|    | TTFA +    | <i>P. cepacia</i>    | <10               | <10               | <10               | <10               |
|    | 0.1%      | <i>P. aeruginosa</i> | <10               | <10               | <10               | <10               |
| 25 | EDTA +    | <i>C. albicans</i>   | <10               | <10               | <10               | <10               |
|    | BAK       |                      |                   |                   |                   |                   |

Table 6 demonstrates a synergy in terms of killing capacity and speed of kill when TTFA is used in combination with BAK against a wide variety of microorganisms, both bacteria and fungi. Note particularly the sixth section of Table 6 where solutions inoculated with  $10^6$  colony forming units of the two tough to kill bacteria, *P. cepacia* and *S. marcescens*, experienced much more than a three log kill within six hours time, and where solutions

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inoculated with  $10^6$  colony forming units of the three USP standard tester strains, *C. albicans*, *P. aeruginosa*, and *S. aureus*, experienced a three log or greater kill within six hours time. Contrasting

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the second and fourth sections of Table 6, where BAK and TTFA in PBS solution, respectively, were tested, with section six of Table 6 demonstrates the synergy of the TTFA and BAK combination with respect to performance of the preservative against *P. cepacia* and *S. aureus* in particular. The seventh section of Table 6 shows that a combination of TTFA, BAK and EDTA provides an effective preservative against all organisms tested. Contrasting sections 1-5 with section 7 of Table 6 dramatically demonstrates the synergy of the combination. While Table 6 shows test results at pH 7, similar results in terms of efficacy and speed of kill of the TTFA and BAK combination were also observed at pH 6 and pH 8 and would be expected within the pH range of pH 5 to pH 9.

Phenanthroline, like TTFA, is a hydrophobic chelator compound. Table 7 demonstrates that when phenanthroline is used alone, or when the quaternary ammonium salts BAK or CPC are used alone, they are ineffective against *P. cepacia* 25416; however, preservative systems having a combination of phenanthroline and BAK or CPC are very effective.

TABLE 7

Antimicrobial Effectiveness of Phenanthroline in combination with BAK or CPC and 0.1% EDTA against *P. cepacia* 25416 in PBS at pH 7

| phenanthroline |      | Benzalkonium chloride (BAK) in ppm |                   |                   |                   |
|----------------|------|------------------------------------|-------------------|-------------------|-------------------|
| % wt/wt        | Time | 0                                  | 5                 | 10                | 20                |
| 0              | 6hr  |                                    | $1.3 \times 10^6$ | $1.2 \times 10^6$ | $1.2 \times 10^6$ |
|                | 1day |                                    | $1.2 \times 10^6$ | $1.2 \times 10^6$ | $1.2 \times 10^6$ |
|                | 6hr  | $1.2 \times 10^6$                  | $3.0 \times 10^4$ | $1.0 \times 10^3$ | <10               |
|                | 1day | $4.4 \times 10^2$                  | <10               | <10               | <10               |

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| phenanthroline |      | Cetylpyridinium chloride(CPC) in ppm |                   |                   |
|----------------|------|--------------------------------------|-------------------|-------------------|
| % wt/wt        | Time | 0                                    | 5                 | 10                |
| 5              | 0    | 6hr                                  | $1.2 \times 10^6$ | $1.5 \times 10^5$ |
|                |      | 1day                                 | $1.2 \times 10^6$ | $5.7 \times 10^5$ |
|                | 0.1  | 6hr                                  | $1.2 \times 10^6$ | $3.3 \times 10^2$ |
|                |      | 1day                                 | $4.4 \times 10^2$ | $<10$             |
|                |      |                                      | $<10$             | $<10$             |

Table 7 shows that when 0.1% phenanthroline is used with as little as 10 ppm BAK or as little as 5 ppm CPC, a three log kill is achieved within six hours for a PBS solution inoculated with  $10^6$  cfu of *P. cepacia*.

Benzyl alcohol has been used as a preservative in health care products such as contact lens solutions. One commercial contact lens solution currently uses 0.1% benzyl alcohol; however, 0.1% benzyl alcohol has virtually no antimicrobial effect against *P. cepacia* after three days exposure, even in the presence of 0.1% EDTA (see Table 8 below). Table 8 also shows that when a quaternary ammonium compound such as benzalkonium chloride is used at 10 or 20 ppm, with 0.1% EDTA, little if any killing of *P. cepacia* occurs in three days, but when benzyl alcohol, EDTA and 20 ppm BAK are used together, more than a three log kill occurs in one day and a complete kill is observed in three days.

Phenylethyl alcohol has not been used as a preservative in health care products such as contact lens solutions. An effect similar to benzyl alcohol is shown in Table 8 with phenylethyl alcohol, except that the killing effect of the combination is even greater. Thus, Table 8 shows that when alcohols such as a benzyl and phenylethyl

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alcohol are used in combination with low concentrations of quaternary ammonium compounds such as BAK, they can provide effective preservative activity.

5

TABLE 8

Antimicrobial Effectiveness of Phenylethyl Alcohol or Benzyl Alcohol and EDTA in Combination with BAK against *P. cepacia* 25416

|    |                     |       |                                    |                   |                   |
|----|---------------------|-------|------------------------------------|-------------------|-------------------|
| 10 | pH 7.0 PBS          |       | Benzalkonium chloride (BAK) in ppm |                   |                   |
|    | alcohol             |       |                                    |                   |                   |
|    | % wt/wt             | Time  | 0                                  | 10                | 20                |
|    | 0                   | 1day  | $1.7 \times 10^6$                  | $7.6 \times 10^5$ | $7.4 \times 10^5$ |
|    |                     | 3days | $2.0 \times 10^6$                  | $1.8 \times 10^6$ | $6.4 \times 10^5$ |
| 15 | Benzyl alcohol      |       |                                    |                   |                   |
|    | 0.1                 | 1day  | $4.1 \times 10^5$                  | $1.9 \times 10^5$ | $4.3 \times 10^2$ |
|    |                     | 3days | $1.0 \times 10^6$                  | $8.7 \times 10^4$ | <10               |
|    | phenylethyl alcohol |       |                                    |                   |                   |
|    | 0.1                 | 1day  | $3.3 \times 10^5$                  | $6.0 \times 10^4$ | <10               |
| 20 |                     | 3days | $1.3 \times 10^5$                  | <10               | <10               |

The data in Tables 1-8 demonstrate a synergistic killing activity when a quaternary ammonium compound is used in combination with a paraben, hydrophobic chelator, or alcohol compound.

25 Test data have been presented for combinations which include either BAK and CPC; however, other related quaternary ammonium compounds and combinations of two or more quaternary ammonium compounds could be used in combination with the paraben, hydrophobic chelator, or alcohol compound. Suitable alternative

30 quaternary ammonium compounds to BAK and CPC include substituted benzalkonium compounds such as alkyl dimethylethylbenzylammonium chloride or heteroatomic ammonium salts, twin chain quats like

35 dioctyl dimethyl ammonium bromide,

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bisquaternary ammonium salts such as triclobisonium chloride, polymeric quaternary ammonium compounds such as polyquaternium, and tri-quaternary phosphate esters such as Monoquat PTC etc. The concentration of the quaternary ammonium compound can vary depending on the compound chosen. For example, some of the polyquats may be effective in the combined preservative system at levels as low as 1-4 ppm, while some monoquats may need to be present in the combined preservative system at levels of 100 ppm or greater. Preliminary data suggest that when Monoquat PTC at a concentration of 100 ppm is combined with methyl paraben, the combined preservative system has a synergistic killing capability for *P. cepacia*. The synergistic microorganism killing activity achieved when used in combination with a paraben, hydrophobic chelator, or alcohol compound allows lower concentrations of the quaternary ammonium compound to be used, thereby reducing user irritation and health risks.

Tables 1-5 demonstrate the efficacy of methyl paraben in the preservative combinations, and it should be understood similar results could be achieved with other parabens such as ethyl, propyl and butyl parabens. Table 6 demonstrates the efficacy of TTFA and Table 7 demonstrates the efficacy of phenanthroline in the preservative combinations. Table 8 demonstrates the efficacy of alcohols such as phenylethyl and benzyl alcohol in the preservative combinations. Other hydrophobic chelator compounds similar to TTFA and phenanthroline, such as methyl, nitro, and

chlorophenanthroline, thenoyltrifluoroacetone, hydroxyquinoline and its derivatives, bipyridine, picolinic acid, and dipicolinic acid, can be used in combination with the quaternary ammonium compound within the practice of this invention.

5 The preservative systems of this invention can be used in aqueous solutions such as contact lens solutions and other eye care products, nasal sprays, mouth washes, medical lavage fluids and disinfectants, shampoos, cold medicines, as well as other saline injectible medicines. These types of health care products typically have a neutral or near neutral pH (e.g., pH 5-pH 9). As can be seen from Tables 1-8, the preservative systems of the present invention are contemplated for use in solutions having a pH ranging from pH 5 to pH 9.

15 While the preservative systems were mainly tested against bacteria, it should be understood that they would be effective against molds and yeasts as demonstrated in Table 6.

20 While the tests shown in Tables 1-8 disclose results when 0.1% EDTA was included in solution, concentrations of EDTA up to 0.5% are used without causing irritation to the user.

25 The following Examples disclose contact lens, eye wash and nasal spray formulations which include the preservative systems contemplated by the present invention and are provided for exemplary purposes only. The Examples should not be construed as limiting the applicability of the preservative systems in other aqueous and nonaqueous formulations.

30



## EXAMPLE 1

A contact lens solution or eye care formulation, including:

5

water;

sodium chloride;

buffering agents such as sodium and potassium phosphate;

10

viscosity increasing agents such as polyvinyl alcohol;

wetting agents;

EDTA;

15

1-50 ppm of a quaternary ammonium compound selected from the group consisting of BAK, CPC, other related quaternary ammonium compounds including substituted benzalkonium compounds such as alkyldimethylethylbenzylammonium chloride or heteroaromatic ammonium salts, twin chain quats like dioctyl dimethyl ammonium bromide, bisquaternary ammonium salts such as triclobisonium chloride, polymeric quaternary ammonium compounds such as polyquaternium, and tri-quaternary phosphate esters such as Monoquat PTC, etc.; and, alternatively

25

0.05-0.3% wt/wt of a paraben selected from the group consisting of methyl, ethyl, propyl and butyl parabens,

0.05-1% wt/wt of an alcohol selected from the group consisting of benzyl and phenylethyl alcohol, or

30

0.05-1% wt/wt of a hydrophobic chelator selected from the group consisting of phenanthroline and its derivatives methyl and chlorophenanthroline, thenoyltrifluoroacetone,

hydroxyquinoline and derivatives, bipyridine, picolinic acid, and dipicolinic acid,

the solution having a pH ranging between pH 5 and pH 9.

5

## EXAMPLE 2

A nasal spray, including:

water;

10 a therapeutically acceptable amount, e.g., 1-20 wt%, of a bronchodilator (albuterol, beclomethasone dipropionate, chromolyn sodium, etc.), an analgesic, an antiinflammatory (e.g., ipratropium, atropine methonitrate, exogenous  
15 opioid agonists, alpha adrenergic agonists, antihistamines including chlorpheniramine, prostaglandin blockers and antagonists, leukotriene blockers and antagonists, parasympathetic blockers and antagonists, interleukin blockers and  
20 antagonists, and nonsteroidal antiinflammatory agents including naproxen and ibuprophen), antiviral (e.g., inteferons, interferon inducers, capsid binding agents, benzoimidazoles, 1'-methyl spiro(adamantane-2,3-pyrrolidine)maleate, isatin  
25 thiosemicarbazone, fusidic acid, substituted trizainoindoles, 2,6-diphenyl-3-methyl-2,3-dihydroimidazo[2,1-b]thiazole, 3-alpha-naphthyl-5-diethylcarbamoyl-1,2,4,-oxadiazole, oxolinic acid, isoquinolines, 1-p-chlorophenyl-3-(m-3-isobutyl-guanidinophenyl)urea hydrochloride, anti ICAM-1  
30 antibody and other viral receptor antibodies, synthetic ICAM-1 and other synthetic viral receptors, amantadine, rimantadine, and ribavirin,

etc.), or steroid (e.g., triamcinolone, etc.) compound;

5 1-50 ppm of a quaternary ammonium compound selected from the group consisting of BAK, CPC, other related quaternary ammonium compounds including substituted benzalkonium compounds such as alkyldimethylethylbenzylammonium chloride or heteroaromatic ammonium salts, twin chain quats like dioctyl dimethyl ammonium bromide, bisquaternary 10 ammonium salts such as triclobisonium chloride, polymeric quaternary ammonium compounds such as polyquaternium, and tri-quaternary phosphate esters such as Monoquat PTC, etc.; and alternatively

15 0.05-0.3% wt/wt of a paraben selected from the group consisting of methyl, ethyl, propyl and butyl parabens,

0.05-1% wt/wt of an alcohol selected from the group consisting of benzyl and phenylethyl alcohol, or

20 0.05-1% wt/wt of a hydrophobic chelator selected from the group consisting of phenanthroline and its derivatives methyl and chlorophenanthroline, thenoyltrifluoroacetone, hydroxyquinoline and derivatives, bipyridine, 25 picolinic acid, and dipicolinic acid,

the solution having a pH ranging between pH 5 and pH 9.

30 In certain nasal sprays, much higher concentrations of the quaternary ammonium compound should be used because of the presence of surfactants which neutralize some of the compound. In such products, the concentration range of the quaternary ammonium compound (BAK, etc.) can be

increased to as high as 500 ppm or more to counteract the neutralizing effect of commonly used surfactants.

5

**EXAMPLE 3**

A cough or cold formulation, including:  
water;  
expectorants including guaifenesin,  
10 antihistamines including chlorpheniramine and  
triprolidine hydrochloride,  
cough suppressants including dextromethorphan  
hydrobromide,  
analgesics including acetaminophen,  
15 decongestants including phenylpropanolamine  
hydrochloride and pseudoephedrine hydrochloride,  
1-50 ppm of a quaternary ammonium compound  
selected from the group consisting of BAK, CPC,  
other related quaternary ammonium compounds  
20 including substituted benzalkonium compounds such  
as alkyldimethylethylbenzylammonium chloride or  
heteroaromatic ammonium salts, twin chain quats like  
dioctyl dimethyl ammonium bromide, bisquaternary  
ammonium salts such as triclobisonium chloride,  
25 polymeric quaternary ammonium compounds such as  
polyquaternium, and tri-quaternary phosphate esters  
such as Monoquat PTC, etc.; and alternatively  
0.05-0.3% wt/wt of a paraben selected from the  
group consisting of methyl, ethyl, propyl and butyl  
30 parabens,  
0.05-1% wt/wt of an alcohol selected from the  
group consisting of benzyl and phenylethyl alcohol,  
or

0.05-1% wt/wt of a hydrophobic chelator  
selected from the group consisting of  
phenanthroline and its derivatives methyl and  
chlorophenanthroline, thenoyltrifluoroacetone,  
5 hydroxyquinoline, bipyridine, picolinic acid, and  
dipicolinic acid,

the solution having a pH ranging between pH 5  
and pH 9.

10 While the invention has been described in  
terms of its preferred embodiments, those skilled  
in the art will recognize that the invention can be  
practiced with modification within the spirit and  
scope of the appended claims.

## CLAIMS

We claim:

- 1        1. A preservative system for a fluid which has a  
2        pH ranging between pH 5 and pH 9, comprising:  
3        1-50 ppm of a quaternary ammonium compound;  
4        and  
5        0.05-1.0% wt/wt of an antimicrobial compound  
6        selected from the group consisting of paraben,  
7        alcohol or hydrophobic chelator compounds, said  
8        quaternary ammonium compound and said antimicrobial  
9        compound providing synergistic antimicrobial  
10       activity in said fluid.
- 1       2. The preservative system of claim 1 wherein said  
2       quaternary ammonium compound is selected from the  
3       group consisting of benzalkonium chloride,  
4       cetylpyridinium chloride,  
5       alkyldimethylethylbenzylammonium chloride, dioctyl  
6       dimethyl ammonium bromide, triclobisonium chloride,  
7       and polyquaternium.
- 1       3. The preservative system of claim 1 wherein said  
2       antimicrobial compound is a paraben compound  
3       selected from the group consisting of methyl  
4       paraben, ethyl paraben, propyl paraben, and butyl  
5       paraben.
- 1       4. The preservative system of claim 1 wherein said  
2       antimicrobial compound is an alcohol compound  
3       selected from the group consisting of benzyl  
4       alcohol and phenylethyl alcohol.

1        5. The preservative system of claim 1 wherein said  
2 antimicrobial compound is a hydrophobic chelator  
3 compound selected from the group consisting of  
4 thenoyltrifluoroacetone, phenanthroline,  
5 methylphenanthroline, chlorophenanthroline,  
6 nitrophenanthroline, hydroxyquinoline, bipyridine,  
7 picolinic acid, and dipicolinic acid.

1        6. The preservative system of claim 1 further  
2 comprising ethylenediaminetetraacetic acid in an  
3 amount ranging between 0.01 and 0.5 % wt/wt.

1        7. The preservative system of claim 1 wherein said  
2 quaternary ammonium compound is selected from the  
3 group consisting of benzalkonium chloride and  
4 cetylpyridinium chloride and the antimicrobial  
5 compound is methyl paraben.

1        8. The preservative system of claim 1 wherein said  
2 quaternary ammonium compound is selected from the  
3 group consisting of benzalkonium chloride and  
4 cetylpyridinium chloride and the antimicrobial  
5 compound is thenoyltrifluoroacetone.

1        9. The preservative system of claim 1 wherein said  
2 quaternary ammonium compound is selected from the  
3 group consisting of benzalkonium chloride and  
4 cetylpyridinium chloride and the antimicrobial  
5 compound is selected from the group consisting of  
6 phenanthroline, methylphenanthroline,  
7 nitrophenanthroline, and chlorophenanthroline.

1        10. The preservative system of claim 1 wherein  
2        said quaternary ammonium compound is selected from  
3        the group consisting of benzalkonium chloride and  
4        cetylpyridinium chloride and the antimicrobial  
5        compound is phenylethyl alcohol.

1        11. A method of preserving fluids having a pH  
2        ranging between pH 5 and pH 9 from contamination by  
3        microorganisms by providing the fluids with both a  
4        quaternary ammonium compound and an antimicrobial  
5        compound selected from the group consisting of  
6        paraben, alcohol or hydrophobic chelator compounds,  
7        said quaternary ammonium compound and said  
8        antimicrobial compound being present at levels  
9        which achieve synergistic microorganism killing  
10       activity.

1        12. A method of killing microorganisms selected  
2        from the group consisting of *A. niger*, *S. aureus*,  
3        *S. marcescens*, *P. cepacia*, *P. aeruginosa*, and *C.*  
4        *albicans*, comprising the step of exposing said  
5        microorganisms to a fluid containing both a  
6        quaternary ammonium compound and an antimicrobial  
7        compound selected from the group consisting of  
8        paraben, alcohol or hydrophobic chelator compounds,  
9        said quaternary ammonium compound and said  
10       antimicrobial compound being present at levels  
11       which achieve synergistic microorganism killing  
12       activity.



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/05693

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A01N 31/00, 33/12, 37/10, 43/40, 43/42

US CL : 514/292, 310, 311, 334, 354, 358, 544, 642, 643, 730

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/292, 310, 311, 334, 354, 358, 544, 642, 643, 730

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|-----------|---|-----------------------|
| Y         | US, A, 4,474,748 (Sipos) 02 October 1984, see entire document.  | 1,2,4,6 and 10-12     |
| Y         | US, A, 2,694,663 (Stayner) 16 November 1954, see entire document.   | 1,2 and 6-12          |
| Y         | Chemical Abstracts, Volume 80, No. 13 issued 01 April 1974, Richards et. al., "Enhancement of benzalkonium chloride and chlorhexidine acetate activity against Pseudomonas aeruginosa by aromatic alcohols", abstract no. 66969f. | 1,2,4,6 and 10-12     |

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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| * Special categories of cited documents:   | * T  | later documents published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention   |
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Date of the actual completion of the international search

02 AUGUST 1994

Date of mailing of the international search report

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## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|-----------|--|-----------------------|
| Y         | Chemical Abstracts, Volume 76, No. 15 issued 10 April 1972, Richards et. al., "Phenylethanol enhancement of preservatives used in ophthalmic preparations", abstract no. 81660j.                                   | 1-4,6,7 and 10-12     |
| Y         | Chemical Abstracts, Volume 113, No. 10 issued 03 September 1990, Morita et. al., "Ophthalmic solutions containing benzalkonium chloride, p-hydroxybenzoate esters, and chelating agents." Abstract no. 113:84881a. | 1-3,6,7, 11, and 12   |
| Y         | Windholz et. al., "The Merck Index", published 1983 by Merck & Co., Inc., (Rahway, N. J., U. S. A.), Tenth Edition, see pages 706 and 707, abstract no. #4765.   | 1,2,5,6,11 and 12     |